FDA/USDA/CDC

National Antimicrobial Susceptibility Monitoring Program - Veterinary Isolates

April, 1998

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INTRODUCTION

The emergence of resistance to antimicrobics has compromised control of many bacterial pathogens and is a global problem. Additionally, multiple resistance has emerged among many bacterial strains including *Salmonella* species. A penta-resistant strain of *Salmonella typhimurium* DT104 in which the resistance genes have been chromosomally integrated is proving to be particularly problematic resulting in increased morbidity and mortality in both animals and humans.

The development of resistant human pathogenic bacteria may result from direct use of antimicrobial agents in humans and animals and acquisition of resistant organisms or resistance factors from animal and environmental bacteria. The intestinal flora of animals that have been exposed to antimicrobial agents can serve as a reservoir of resistant bacteria.

Because of the public health concerns associated with the use of antimicrobics in food-producing animals, an antimicrobial resistance monitoring program was proposed by the Food and Drug Administration Center for Veterinary Medicine (FDA) as a post-marketing activity to help ensure the continued safety and efficacy of veterinary antimicrobics. In 1996, the CDC, the USDA, and the FDA established the National Antimicrobial Susceptibility Monitoring System to prospectively monitor changes in antimicrobial susceptibilities of zoonotic pathogens from human and animal clinical specimens, from healthy farm animals, and from carcasses of food-producing animals at slaughter. Non-typhoid *Salmonella* was selected as the sentinel organism.

Veterinary testing is conducted at USDA's Agricultural Research Service Russell Research Center in Athens, GA. Testing is done using a semi-automated system (Sensititre™ Accumed, Westlake Ohio). This report summarizes the percentage of isolates collected during calendar year 1997 that were susceptible, intermediate, or resistant to 17 antimicrobics (n=2,391). The 17 antimicrobics were chosen to be representative of common antimicrobics (or classes of antimicrobics) used in animal and human medicine. A subsequent report will summarize the minimal inhibitory concentrations obtained for these isolates and will provide a discussion of the data. Questions regarding this report should be directed to any of the people listed below.

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GOALS AND OBJECTIVES

The goals and objectives of the monitoring program are to:

1) provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and other enteric organisms from the human and animal populations;

2) facilitate the identification of resistance in humans and animals as it arises;

3) provide timely information to veterinarians and physicians;

4) prolong the life span of approved drugs by promoting the prudent and judicious use of antimicrobics; and

5) identify areas for more detailed investigation.

Information resulting from the monitoring program and follow-up outbreak investigations will be distributed to veterinarians, physicians, and food animal producer groups. Use of the information will be targeted to redirecting drug use so as to diminish the development and spread of resistance over the short term with directives involving long-term use developed in collaboration with the appropriate professional practitioner groups. Outbreak investigations and field studies will be initiated as a result of major shifts or changes in resistance patterns in either animal or human isolates.

METHODOLOGY

Isolation:

Salmonella isolates with known serotypes are struck onto 5% sheep blood agar (SBA) plates for isolation. Plates are incubates at 37°C overnight. The following morning one well-isolated colony from each plate is picked and regrown on a second SBA plate which is incubated at 37°C overnight.

Screening for resistance:

One sterile dd H_20 tube and 1 Mueller- Hinton broth (MHB) tube is set in a rack for each isolate. One substrate strip is added to each MHB for a minimum of 15 minutes prior to inoculation (Note: Once substrate strips are added to MHB tubes, they must be used within 1 hour or discarded). Two to six colonies from the second SBA are collected with a sterile cotton tipped swab and used to inoculate the water tube. The tube is vortexed and the density is adjusted with the Nephlometer as per manufacturer's instructions (Note: the machine is calibrated with a McFarland standard prior to starting the procedure). A 10 µl disposable loop from Sensititre is used to transfer 10 µl from the inoculated water to a MHB tube containing the substrate strip. The MHB tube is vortexed and placed into the auto inoculator (typically one isolate per microtiter plate) as per manufacturer's instructions. The microtiter plate is incubated at 37°C for 18 - 20 hours (Note: The time for reading plates is 18-20 h, ideally all plates are read as close to 18 hrs. as possible). Record the time the microtiter plate is inoculated and read on a sheet. Do NOT read plates or keep data from plates >20 h old). (Note: Ideally plates should not be stacked while in the incubator. If stacking is required, stack no more than 2 plates high.)

Microtiter plates are read as per manufacturer's instructions

Freezing clones:

Using a sterile disposable 1 µl inoculating loop 6 colonies from the second SBA plate are picked and inoculated (by vigorously shaking the loop to dislodge bacteria) into 1 ml LB broth plus 30% glycerol in cryo vials. The vials are stored frozen at -70°C and labeled with the following information:

Information	Example
SURVEY	AB CLONE
STUDY	FSIS 95
ISOLATE #	2345
DATE	2/26/96

TABLE OF ANTIMICROBICS

Antimicrobic	Antimicrobic Concentrations (ug/ml)*	Breakpoint
Amikacin	4 - 32	64
Amoxicillin/Clavulanic Acid	0.5/0.25 - 32/16	32/16
Ampicillin	2 - 64	32
Apramycin	2 - 16	32
Ceftiofur	0.5 - 16	8
Ceftriaxone	0.25 - 16	64
Cephalothin	1 - 32	32
Chloramphenicol	4 - 32	32
Ciprofloxacin	0.015 - 2	4
Gentamicin	0.25 - 16	16
Kanamycin	16 - 64	64
Nalidixic Acid	4 - 64	32
Streptomycin	32 - 256	64
Sulfamethoxazole	128 - 512	512
Tetracycline	4 - 64	16
Ticarcillin	2 - 128	128
Trimethoprim/ Sulfamethoxazole	0.12/2.4 - 4/76	4/76

* ranges were chosen to detect incremental changes in resistance based on previous 2 year data; ranges may be outside of the breakpoint value

RESULTS -
Veterinary Isolates

TABLE 1. Top 15 Salmonella serotypes identified for 1997 (N=2,391 total isolates) for all animal species

Serotype	Serogroup	Frequency (n)	Percent of Total
Montevideo	C1	221	9.2
Kentucky	C3	177	7.4
Typhimurium (copenhagen)*	B	171	7.2
Anatum	E4	169	7.1
Typhimurium*	B	157	6.6
Heidelberg	B	146	6.1
Agona	B	141	5.9
Cerro	K	116	4.9
Mbandaka	C1	92	3.8
Muenster	E1	89	3.7
Derby	B	70	2.9
Worthington	G2	62	2.6
Menhaden	E3	61	2.6
Meleagridis	E1	57	2.4
Hadar	C2	56	2.3

* typhimurium and typhimurium (copenhagen) isolates combined account for 328 (13.7%) of the total number of isolates

TABLE 2: Distribution of isolates by species and clinical status

CLINICAL (isolates collected from NVSL; N=763)

Species	Total Number
Cattle	183
Swine	195
Chicken	153
Exotic	65
Turkey	49
Dog	38
Horse	52
Cat	28

NONCLINICAL (N=1,628)

Species	Number
Cattle	859*
Swine	226
Cattle feed	2
Swine feed	20
HACCP**	**521**
Chicken	223
Turkey	153
Swine	110
Cattle	27
Egg	6
Misc***	2

*includes 99 samples which are of unidentified clinical status **samples collected from carcasses at

slaughter with the exception of eggs ***species unknown

TABLE 3: Total percent sensitive, intermediate or resistant

	Susceptible		Intermediate		Resistant	
Antimicrobic	**n**	**%**	**n**	**%**	**n**	**%**
Amikacin	2391	100	0	0	0	0
Amoxicillin/Clavulanic Acid	2215	92.6	134	5.6	42	1.8
Ampicillin	2104	88.0	0	0	287	12.0
Apramycin	2345	98.1	4	0.2	42	1.8
Ceftiofur	2367	99.0	2	0.1	22	0.9
Ceftriaxone	2374	99.3	11	0.5	6	0.3
Cephalothin	2281	95.4	54	2.3	56	2.3
Chloramphenicol	2273	95.1	8	0.3	110	4.6
Ciprofloxacin	2391	100	0	0	0	0
Gentamicin	2211	92.5	40	1.7	140	5.9
Kanamycin	2145	89.7	4	0.2	242	10.1
Nalidixic Acid	2372	99.2	0	0	18	0.8
Streptomycin	1970	82.4	0	0	421	17.6
Sulfamethoxazole	1968	82.3	0	0	423	17.7
Tetracycline	1727	72.2	8	0.3	656	27.4
Ticarcillin	2109	88.2	4	0.2	278	11.6
Trimethoprim/ Sulfamethoxazole	2341	97.9	0	0	50	2.1

TABLE 4: Percent total resistance by species/sources (includes both clinical and nonclinical isolates)

Antimicrobic	SPECIES Cattle n=1,069	Swine n=531	Chicken n=376	Turkey n=202	Horse n=52
Amikacin	0	0	0	0	0
Amoxicillin/Clavulanic Acid	1.2	0.6	1.1	7.9	3.8
Ampicillin	9.4	12.4	11.2	19.3	19.2
Apramycin	0.1	6.6	0	2.0	0
Ceftiofur	0.1	0.4	0.3	6.4	1.9
Ceftriaxone	0.1	0	0	2.5	0
Cephalothin	1.6	0.8	2.4	8.9	7.7
Chloramphenicol	2.4	7.7	2.4	7.9	9.6
Ciprofloxacin	0	0	0	0	0
Gentamicin	0.4	4.9	14.6	24.3	7.7
Kanamycin	6.6	13.2	3.5	28.7	19.2
Nalidixic Acid	0	0	0.3	8.4	0
Streptomycin	9.7	22.8	22.3	38.6	17.3
Sulfamethoxazole	8.4	23.2	22.6	42.1	21.2
Tetracycline	14.0	49.9	18.1	59.4	23.1
Ticarcillin	8.9	12.4	11.2	18.3	19.2
Trimethoprim/ Sulfamethoxazole	0.7	5.3	1.1	2.5	9.6

TABLE 4: Percent total resistance by species/sources (includes both clinical and nonclinical isolates; continued)

Antimicrobic	Cattle Feed n=2	Swine Feed n=20	SPECIES Exotic n=65	Dog n=38	Cat n=28	Egg n=6
Amikacin	0	0	0	0	0	0
Amoxicillin/Clavulanic Acid	0	5.0	0	0	10.7	0
Ampicillin	0	5.0	3.1	31.6	53.6	0
Apramycin	0	5.0	0	0	3.6	0
Ceftiofur	0	0	0	0	10.7	0
Ceftriaxone	0	0	0	0	0	0
Cephalothin	0	5.0	0	0	10.7	0
Chloramphenicol	0	0	0	13.2	28.6	0
Ciprofloxacin	0	0	0	0	0	0
Gentamicin	0	5.0	0	0	0	0
Kanamycin	0	10.0	3.1	18.4	32.1	0
Nalidixic Acid	0	0	0	0	0	0
Streptomycin	0	20.0	3.1	23.7	35.7	0
Sulfamethoxazole	0	5.0	3.1	31.6	50.0	0
Tetracycline	0	35.0	6.2	36.8	57.1	0
Ticarcillin	0	0	3.1	31.6	50.0	0
Trimethoprim/ Sulfamethoxazole	0	0	1.5	0	0	0

Note: 2 isolates that were unidentified were not resistant to any antimicrobic

Table 5: Percent resistance for non-clinical isolates (excluding HACCP samples)

Antimicrobic	Cattle n=760	Swine n=226
Amikacin	0	0
Amoxicillin/Clavulanic Acid	1.1	1.0
Ampicillin	4.1	1.3
Apramycin	0	11.1
Ceftiofur	0	0.4
Ceftriaxone	0	0
Cephalothin	1.7	1.0
Chloramphenicol	1.6	0
Ciprofloxacin	0	0
Gentamicin	0.1	6.6
Kanamycin	1.7	8.4
Nalidixic Acid	0	0
Streptomycin	4.1	7.5
Sulfamethoxazole	2.9	1.0
Tetracycline	8.0	27.9
Ticarcillin	3.6	1.3
Trimethoprim/ Sulfamethoxazole	0.1	0

Note: Samples are included only if they were able to be identified as non-clinical. This does not include 99 samples of undetermined clinical status

Table 6: Percent resistance for HACCP samples

Antimicrobic	SPECIES Cattle n=27	Swine n=110	Chicken n=223	Turkey n=153	Egg n=6
Amikacin	0	0	0	0	0
Amoxicillin/Clavulanic Acid	7.4	0	0.4	7.2	0
Ampicillin	22.2	15.5	11.7	13.7	0
Apramycin	0	2.7	0	0.7	0
Ceftiofur	0	0.9	0.4	5.9	0
Ceftriaxone	0	0	0	2.0	0
Cephalothin	0	0.9	1.3	7.8	0
Chloramphenicol	14.8	10.0	2.7	5.9	0
Ciprofloxacin	0	0	0	0	0
Gentamicin	0	1.8	17.9	18.3	0
Kanamycin	7.4	12.7	2.2	26.8	0
Nalidixic Acid	0	0	0	5.2	0
Streptomycin	22.2	26.4	24.7	35.9	0
Sulfamethoxazole	33.3	31.8	26.0	36.6	0
Tetracycline	37.0	50.0	21.5	56.2	0
Ticarcillin	22.2	15.5	11.7	13.7	0
Trimethoprim/ Sulfamethoxazole	3.7	1.8	0.9	3.3	0

Table 7: Percent resistance for clinical isolates*

Antimicrobic	SPECIES Cattle n=183	Swine n=195	Chicken n=153	Turkey n=49	Horse n=52
Amikacin	0	0	0	0	0
Amoxicillin/Clavulanic Acid	1.6	0.5	2.0	10.2	3.8
Ampicillin	32.2	23.6	10.5	36.7	19.2
Apramycin	0.5	3.6	0	6.1	0
Ceftiofur	0.5	0	0	8.2	1.9
Ceftriaxone	0.5	0	0	4.1	0
Cephalothin	1.6	0.5	3.9	12.2	7.7
Chloramphenicol	5.5	15.4	2.0	14.3	9.6
Ciprofloxacin	0	0	0	0	0
Gentamicin	2.2	4.6	9.8	42.9	7.7
Kanamycin	29.0	19.0	5.2	34.7	19.2
Nalidixic Acid	0	0	0.7	18.4	0
Streptomycin	33.9	38.5	19.0	46.9	17.3
Sulfamethoxazole	30.6	44.1	17.6	59.2	21.2
Tetracycline	36.6	75.4	13.1	69.4	23.1
Ticarcillin	31.7	23.6	10.5	32.7	19.2
Trimethoprim/ Sulfamethoxazole	1.6	13.3	1.3	0	9.6

Note: Clinical isolates in Table 7 were all obtained from the National Veterinary Services Laboratories, Ames, IA

Table 7: Percent resistance for clinical isolates* (continued)

Antimicrobic	SPECIES Exotic n=65	 Dog n=38	 Cat n=28
Amikacin	0	0	0
Amoxicillin/Clavulanic Acid	0	0	10.7
Ampicillin	3.1	31.6	53.6
Apramycin	0	0	3.6
Ceftiofur	0	0	10.7
Ceftriaxone	0	0	0
Cephalothin	0	0	10.7
Chloramphenicol	0	13.2	28.6
Ciprofloxacin	0	0	0
Gentamicin	0	0	0
Kanamycin	3.1	18.4	32.1
Nalidixic Acid	0	0	0
Streptomycin	3.1	23.7	35.7
Sulfamethoxazole	3.1	31.6	50.0
Tetracycline	6.2	36.8	57.1
Ticarcillin	3.1	31.6	50.0
Trimethoprim/ Sulfamethoxazole	1.5	0	0

Note: Clinical isolates in Table 7 were all obtained from the National Veterinary Services Laboratories, Ames, IA

Table 8: Percent total resistance for the top 15 Salmonella serotypes from animal species/sources

Antimicrobic	Montevi. n=221	Kentucky n=177	SEROTYPE Typh(cop) n=171	Anatum n=169	Typhim. n=157
Amikacin	0	0	0	0	0
Amoxicillin/Clavulanic Acid	0.5	1.7	4.7	0.6	7.0
Ampicillin	2.3	2.8	84.2	0.6	35.0
Apramycin	0	0	1.8	10.1	1.3
Ceftiofur	0.9	0	4.1	0	4.5
Ceftriaxone	0	0	1.2	0	1.3
Cephalothin	1.4	1.7	4.7	0.6	5.7
Chloramphenicol	0	0	36.8	0.6	20.4
Ciprofloxacin	0	0	0	0	0
Gentamicin	2.7	1.7	4.1	5.3	8.3
Kanamycin	1.8	1.7	49.7	0.6	19.1
Nalidixic Acid	0	0.6	2.9	0	1.9
Streptomycin	1.8	12.4	70.8	3.0	33.8
Sulfamethoxazole	1.8	3.4	82.5	3.6	37.6
Tetracycline	0.9	13.6	90.1	47.3	35.0
Ticarcillin	1.4	2.8	84.2	0.6	33.8
Trimethoprim/ Sulfamethoxazole	0	1.1	4.7	0	3.8

Table 8: Percent total resistance for the top 15 Salmonella serotypes from animal species/sources (continued)

Antimicrobic	Heidel. n=146	Agona n=141	SEROTYPE Cerro n=116	Mbandaka n=92	Muenster n=89
Amikacin	0	0	0	0	0
Amoxicillin/Clavulanic Acid	1.4	0.7	0.9	0	0
Ampicillin	15.1	2.8	0.9	2.2	2.2
Apramycin	6.2	0	0	0	0
Ceftiofur	0	0.7	0	0	0
Ceftriaxone	0	0	0	0	0
Cephalothin	3.4	1.4	0.9	1.1	2.2
Chloramphenicol	0.7	0	0	0	0
Ciprofloxacin	0	0	0	0	0
Gentamicin	27.4	1.4	0	1.1	13.5
Kanamycin	31.5	5.0	0	2.2	13.5
Nalidixic Acid	0	0.7	0	0	0
Streptomycin	45.9	4.3	0	2.2	12.4
Sulfamethoxazole	30.1	15.6	0	25.0	12.4
Tetracycline	34.9	25.5	12.1	33.7	19.1
Ticarcillin	15.1	2.1	0	2.2	2.2
Trimethoprim/ Sulfamethoxazole	0.7	0	0	26.1	0

Table 8: Percent total resistance for the top 15 Salmonella serotypes from animal species/sources (continued)

Antimicrobic	Derby n=70	Worthing. n=62	SEROTYPE Menhaden n=61	Meleagrid. n=57	Hadar n=56
Amikacin	0	0	0	0	0
Amoxicillin/Clavulanic Acid	0	3.2	1.6	0	1.8
Ampicillin	5.7	1.6	0	0	10.7
Apramycin	7.1	1.6	0	0	0
Ceftiofur	0	0	0	0	0
Ceftriaxone	0	0	0	0	0
Cephalothin	0	1.6	1.6	0	5.4
Chloramphenicol	4.3	0	0	0	0
Ciprofloxacin	0	0	0	0	0
Gentamicin	5.7	3.2	0	0	10.7
Kanamycin	10.0	0	0	0	16.1
Nalidixic Acid	0	0	0	0	1.8
Streptomycin	51.4	8.1	0	0	51.8
Sulfamethoxazole	48.6	4.8	0	0	12.5
Tetracycline	58.6	21.0	0	0	89.3
Ticarcillin	2.7	0	0	0	10.7
Trimethoprim/ Sulfamethoxazole	1.4	1.6	0	0	0

Table 9: Multiple antimicrobial resistance

Number of Antimicrobics Resistant to	No. Isolates	Percent
0	1572	65.7
1	225	9.4
2	132	5.5
3	147	6.1
4	50	2.1
5	82	3.4
6	130	5.4
7	21	0.9
8	4	0.2
9	9	0.4
10	5	0.2
11	7	0.3
12	5	0.2
13	2	0.1

Table 10: Most frequent resistance patterns

Antimicrobics	No. Isolates	Percent
Tet	177	7.4
Amp/Kan/Strep/Sulfa/Tet/Tic	79	3.3
Strep/Sulfa/Tet	36	1.5
Amp/Chlor/Strep/Sulfa/Tet/Tic	36	1.5
Strep/Tet	34	1.4
Amp/Chlor/Sulfa/Tet/Tic	30	1.3
Kan/Strep/Tet	27	1.1
Gen/Strep/Sulfa	25	1.0
Sulfa/Tet	24	1.0

Table11: Most frequent resistance patterns for 5 or more antimicrobics

Antimicrobics	No. Isolates	Percent
Amp/Kan/Strep/Sulfa/Tet/Tic	79	3.3
Amp/Chlor/Strep/Sulfa/Tet/Tic	36	1.5
Amp/Chlor/Sulfa/Tet/Tic	30	1.3
Gen/Kan/Strep/Sulfa/Tet	14	0.5
Amp/Strep/Sulfa/Tet/Tic	8	0.3
Apra/Gen/Kan/Strep/Tet	8	0.3
Amp/Chlor/Kan/Strep/Sulfa/Tet/Tic	6	0.3
Amp/Gen/Strep/Sulfa/Tic	5	0.2
Amp/Kan/Strep/Sulfa/Tic	5	0.2
Amp/Kan/Strep/Tet/Tic	5	0.2
Amp/Kan/Strep/Sulfa/Tet/Tic/Trisulfa	4	0.2
Amp/Apr/Chlor/Gen/Kan/Strep/Sulfa/Tet/Tic	4	0.2

Table 12: Total S. typhimurium percent resistance with ACSSuT pattern

Serotype	No. Isolates	No. ACSSuT	Percent ACSSuT	Percent of Total (n=2391) ACSSuT
S. typhimurium	157	26	16.6	1.1
S. typhimurium (cop)	171	32	18.7	1.3
Total	328	58	17.7	2.4